IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA,	)	
	)	
Plaintiff,	)	
	)	
v.	)	**Case No. 05-cv-329-GKF(PJC)**
	)	
TYSON FOODS, INC., et al.,	)	
	)	
Defendants.	)	

DECLARATION OF VALERIE J. HARWOOD, Ph.D.

I, Valerie J. Harwood, Ph.D., hereby declare as follows:

1. My terminal degree is a Ph.D. in Biomedical Sciences from Old Dominion University & Eastern Virginia Medical School in Norfolk, VA (1992). From 1992 to 1995 I held a full-time postdoctoral research position at the University of Maryland Center of Marine Biotechnology. In 1995 I joined the Department of Natural Sciences at the University of North Florida as a tenure-track Assistant Professor, where I taught microbiology and related courses, and maintained a research laboratory until I joined the University of South Florida (USF) in Tampa, FL in August 1998. Since that time I have been employed by USF in the Department of Biology (now the Department of Integrative Biology) in a full-time position. In 2004 I was promoted to Associate Professor, which is my current rank, and was awarded tenure. My responsibilities at USF include teaching undergraduate and graduate courses in microbiology, mentoring undergraduate and graduate research students, and maintaining an active research program. My research laboratory personnel currently include one technician and six Ph.D. students. My research focuses on microbial water quality, with particular emphasis on microbial source tracking (MST), a field of environmental microbiology that seeks to determine the source of fecal contamination in water by identifying specific molecular signatures in the DNA of fecal microorganisms.

2. I have worked in the field of environmental microbiology since 1986, and in the area of MST since 1997. I am the author of 34 peer-reviewed journal articles and

three peer-reviewed, published reports, twelve of which are directly related to MST. One of these articles has been cited in other peer-reviewed publications 121 times to date (100 citations is an important benchmark that few papers reach). Other publications include over 30 technical reports, a book chapter, and substantial contributions to the U.S. Environmental Protection Agency Microbial Source Tracking Guide Document. I am also co-editor of a book on microbial source tracking that is contracted to be published by Springer Scientific Press in 2010, and I have been an invited speaker on water quality research and MST over 50 times across the U.S., in the U.K. and in New Zealand. I am a reviewer for many scientific journals including Environmental Science & Technology, Environmental Microbiology, and Journal of Applied Microbiology, and am a member of the editorial review board of Applied & Environmental Microbiology. I have served on state and federal grant panels including Sea Grant, National Oceanic and Atmospheric Administration (NOAA) and the United States Department of Agriculture (USDA), and have been awarded over $3 million in grant funding from various agencies including the National Science Foundation, NOAA, Sea Grant, USDA, United States Environmental Protection Agency (USEPA) and National Institutes of Health. My current funding for MST and related environmental microbiology research totals over one million dollars from agencies including the Florida Department of Environmental Protection, the Water Environment Research Foundation, the US Department of Agriculture and the US Environmental Protection Agency.

3. I have studied the defendants' Motion to Exclude Expert Testimony Based on Bacterial Analyses Conducted in Violation of EPA, USGS and Oklahoma Standards. My expert opinion described herein applies to the reliability of analysis conducted on samples collected by CDM for the Oklahoma Attorney General's office in the Illinois River watershed (IRW). My expert opinion is that the analysis of these samples after holding times of greater than six hours provides reliable and valuable information on the microbiological quality of these waters. The reasons for this opinion are outlined below and detailed in the body off this affidavit.

- These data provided additional information about the extent of bacterial contamination in the IRW (in addition to State of Oklahoma and USGS data);

- Samples were shipped on ice on ice and held below 10º C until they were set up for analysis in the laboratory, in accordance with recommended microbiological practices;
- Although most analyses were set up (sample placed in nutrient broth to begin the analysis) 24 hours or more after the samples were collected, such a delay would lead to decreased estimates of bacterial numbers rather than increased estimates. Thus, the analytical results obtained for indicator bacteria concentrations in these samples would tend to err (if anything) on the side of underestimation of bacterial contamination of IRW waters, rather than overestimation.

4. The purpose of analyzing the IRW water samples was to add to existing data collected by the State of Oklahoma and the USGS on the extent of bacterial contamination in IRW waters and the percentage of samples that exceeded State and federal water quality guidelines (Teaf, 2008). Due to lack of a reliable analytical laboratory with proximity to the study site, samples were shipped on ice by overnight freight to analytical laboratories. The vast majority of these samples began their analysis within 24 to 30 h of sample collection (see Dr. Olsen's affidavit), which was as rapid as possible given the shipping requirement. It should be noted that all care was taken to ensure that the samples remained cold and that they arrived as quickly as possible at the laboratory.

5. *E. coli*, enterococci and pathogens are living things that suffer negative consequences when they pass from the preferred gastrointestinal tract habitat to the water, which is too low in nutrients for them to grow or to maintain their metabolism indefinitely. Sampling and analytical procedures for environmental samples include beginning the analysis (proceed to set-up) as soon as possible in order to avoid die-off of the organisms; in other words, the holding time should be minimized as much as logistically possible. Because microorganisms are very small, and analyses done with microscopes are very labor-intensive, the general strategy in analysis of pathogens and indicator bacteria is to allow the organisms to grow for a period of time (usually 24-48 hours) so that a visual check of their growth is possible. This is called culture-based or culture-dependent analysis, and has been the standard for a century (only in the last two

decades are culture-dependent methods being augmented or supplemented by PCR-based, culture-independent methods). Many standard methods for pathogen or indicator analysis require several culture-based steps that each require 1-2 days to perform, therefore the total analytical time required to confirm results can stretch out for over two weeks. It is important to understand that the hold time or set-up time (from sample collection to inoculation of the sample in the first culture medium) is the crucial time period for insuring that one does not *underestimate* the concentration of target microorganisms in the sample.

6. Regulatory agencies generally stipulate a maximum 6 hour hold time for microbiological analysis of surface water samples (State of Oklahoma, 2006; U.S. Environmental Protection Agency, 2000). This recommendation is based largely on a study conducted in 1953 (The Public Health Laboratory Service Water Sub-Committee, 1953), and is stipulated for samples that are taken for regulatory (compliance) purposes, e.g. beach water quality monitoring or assessment of ambient water quality for TMDL programs. The stipulation is made because bacteria tend to die off in samples that are held for long periods. Thus, the effect of extended holding time is that bacterial concentrations will be *lower* than if the analysis was done immediately. Most studies, however, have found that either no significant differences from the 6 hour holding time results when samples are held 24-48 hours at 8 - 10° C (refrigerated or on ice), or decreases in bacterial concentrations (Selvakumar et al., 2004; Standridge & Lesar, 1977; The Public Health Laboratory Service Water Sub-Committee, 1953). For example, the study on which U.S. EPA and USGS regulations are based found that 21.5% of samples that were tested for fecal coliforms after 24 hours of refrigeration decreased in concentration, while only 3.5% of samples showed an increased concentration (The Public Health Laboratory Service Water Sub-Committee, 1953). The great majority of samples (75%) showed no change. Because fewer samples showed a change when held for 6 hours compared to 24 hours (the only two times tested), the authors recommended the 6 hour holding time. In the words of the authors:

> "There is a much greater probability (1 in 20) of a large change, particularly in the direction of a decrease, in either the coliform or faecal *coli* content of a sample if it is stored for 24 hr at either room or refrigerator temperature before the test is set up."

7. Other studies have corroborated these findings; water samples held at refrigerator temperatures for 24 or up to 48 hours experience no change, or a decrease in bacterial concentrations (Pope et al., 2003; Selvakumar et al., 2004). Standard Methods for the Examination of Water and Wastewater also stipulates that ambient water samples collected for non-regulatory purposes can be held for 24 hours at cold temperatures before analysis (Sec 9060B) (American Public Health Association, 2005).

8. Standridge and Lesar (1977) present a detailed literature review and an experimental holding time study for fecal coliforms. They noted that previous studies on holding times performed to that date had produced conflicting results, and they devised a study that included a strong statistical analysis. Their finding was that the 24 hour holding time for samples analyzed for fecal coliforms produced equivalent results to a 4 hour holding time. Longer holding times were not analyzed in the Standrige and Lesar work. Holding time studies performed in my laboratory corroborate their results; fresh water samples held for approximately 24 hours on ice or in the refrigerator generally have unchanged bacterial concentrations compared to their counterparts held 6 hours or less; if anything, bacterial concentrations decreased with holding time. Enterococci concentrations did not change significantly with a 48 hour hold time.

9. My expert opinion, based upon the literature reports and work conducted in my laboratory, is that the data from the samples collected for the State by CDM are useful and scientifically valid for assessing the extent of microbial contamination in IRW waters. If anything, the data from samples held longer than 30 hours will tend to underestimate the microbial contamination in these waters, particularly for *E. coli* and fecal coliform concentrations. The enterococci concentrations should not change significantly even with a 48 hour holding time, and any changes that occur with longer hold times should be a decrease in concentration. The data collected for this study do not exaggerate microbial contamination and associated human health risks in the IRW. If anything, they are an underestimate due to the tendency of indicator bacteria to die off with increased holding times.

10. Please note that my opinions in this matter are my own, and do not reflect an official view of the University of South Florida.

I declare under penalty of perjury, under the laws of the United States of America, that the foregoing is true and correct.

Executed on the 4th day of June, 2009.

Valerie J. Harwood, Ph.D.

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